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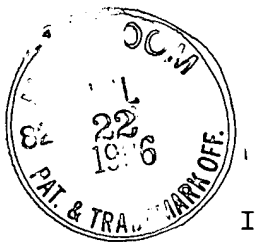

#9 8/23/96

PATENT DOCKET 175C2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	Examiner: S. Priebe
Arjun Singh	)	
	)	Group Art Unit: 1805
Serial No. 08/448,946	)	
	)	
Filed: 24 MAY 1995	)	
	)	
For: USE OF ALPHA FACTOR SEQUENCES IN YEAST EXPRESSION SYSTEMS	)	
	)	

DECLARATION REGARDING AMENDATORY MATERIAL
UNDER MPEP §608.01(p)(B)

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

I, Janet E. Hasak, do declare and say as follows:

1. I am the attorney of record in the above-identified application.

2. The material from U.S. Ser. No. 06/438,128 that is being inserted by the accompanying amendment into pages 8, 21, and 32 and the insertion of Figures 13, 14, 15A, 15B, and 16 into the above-identified application consist of the same material incorporated by reference in the above-identified referencing application, with the exceptions that obvious typographical errors have been corrected (such as "and" instead of "an" and "$MgCl_2$" instead of "MgCl2") and text on other subclones than that referenced and a Table I have been omitted as irrelevant. Further, the reference numbers are renumbered from references 50-54, 8, 9, 12, and 55-68 to references 63-84 respectfully and consecutively to conform with the numbering of references after reference 62 of the instant application, and the figure numbers are renumbered from Figures 1, 2, 3A, 3B, and 4 to Figures 13, 14, 15A, 15B, and 16, respectively, to conform with the numbering

of figures after Figure 12 of the instant application.

3. The material from U.S. Ser. No. 06/452,227 that is being inserted by the accompanying amendment into pages 8, 22, and 32 and the insertion of Figure 17 into the above-identified application consist of the same material incorporated by reference in the above-identified referencing application, with the exceptions that obvious typographical errors have been corrected (such as "ml" instead of "mls" and "more than" instead of "more") and the figure number is renumbered from Figure 1A to Figure 17, to conform with the numbering of figures after Figure 12 of the instant application and Figures 13-16 from the other referenced patent application.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: July 16, 1996

Janet E. Hasak
Janet E. Hasak
Reg. No. 28,616

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

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Kenneth M Goldman
(Signature of Person Mailing Paper or Fee)

BRAKE	:	
v.	:	Interference No. 102,728
SINGH	:	Examiner-in-Chief: R. Smith

DECLARATION OF ANTHONY J. BRAKE

Box Interference
Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

I, Anthony J. Brake, hereby declare:

1. I hold a Ph. D. in Biological Chemistry from the University of California, Los Angeles, and have over thirteen years of experience in molecular biology and recombinant DNA technology. I have authored or coauthored over 20 scientific publications. I am currently on a leave of absence from Chiron Corporation, which leave began in June 1991, when I was director of Yeast Biology. I began employment at Chiron on February 1, 1982. I am the inventor of the subject matter claimed in U.S. Patent No. 4,870,008. A copy of my *curriculum vitae* is submitted herewith as Exhibit 20.

2. I have read and understand the claims in the Singh application, U.S. Serial No. 552,719, and the Brak patent applications, U.S. Serial Nos. 457,325 ("Brake 1") and 522,909 ("Brake 2"). I have also read and understand the Count in this interference.

3. My review of the Brake 1 patent application indicates that it clearly discloses Saccharomyces α-factor constructs for secretory expression of heterologous genes, which constructs lack a dipeptidylaminopeptidase A ("DPAP A") site. This is disclosed, inter alia, in Brake 1 on page 3, line 30 to page 6, line 15.

4. My review of the Brake 1 patent application also indicates that it clearly discloses all embodiments of my invention known to me at the time of filing. Thus, the disclosure must have included the best mode contemplated by me for practicing my invention. A plasmid, pYEGF-8, disclosed in the application and containing the best DNA construct known to me and in my possession at the time of filing of Brake 1, was deposited at the ATCC on January 5, 1983.

5. As of January 1983 there were established and well known techniques available to persons of ordinary skill in the art by which constructs such as the one set forth in the Count could easily have been made:

a. For example, such constructs could have been made using site specific mutagenesis, a technique extensively used by January 1983 to modify DNA. This technique could have been

performed on (1) the construct ex mplified and deposited in the Brake 1 application, or (2) a similar construct made per the description in Brake 1. The technique of site specific mutagenesis (disclosed in Brake 2 and used to make constructs within the Count as shown at page 16, line 22 through page 18, line 16) was available in January 1983, and it would have been apparent to one of ordinary skill at that time to apply the technique to the material disclosed in Brake 1 to produce a construct of the Count.

b. An alternative technique would have been to digest the disclosed vector in Brake 1 with the restriction enzyme Hind III and then treat the digest with Bal 31. This technique would "chew" back the α-factor leader sequence to remove the Glu (or Asp)-Ala codons. One could easily have screened the Bal 31 digested material and isolated a fragment lacking the Glu (or Asp)-Ala codons that appear in the DPAP A site. Then the fragment would be blunt-end ligated to a foreign gene sequence using a suitable adaptor lacking the DPAP A site to form the spacerless construct of the count.

6. Once one constructed a construct lacking a DPAP A site, one of ordinary skill in the art would have recognized that it would be used in the identical way as the construct exemplified in the Experimental section starting on page 12 of Brake 1.

7. In 1983, I attended the 12th Annual UCLA Symposia, which were held between March 27 and April 30, 1983 in Keystone,

Colorado. On April 29, I gave a poster session and presentation in which I presented a series of *S. cerevisiae* α-factor constructs, including spacerless constructs, such as the ones described in Brake 2 and exemplified by the Count in the present interference.

8. During the April 29 poster session, I presented the results of my experiments demonstrating the successful construction of a yeast expression vehicle including the DNA construct embodied in claim 8 of the Singh application.

9. One construct in particular consisted of the *S. cerevisiae* α-factor leader sequence, terminating with the sequence encoding the first Lys-Arg dipeptide, connected in translation reading frame to the sequence encoding the first amino acid of mature epidermal growth factor (EGF). This DNA construct encoded a Lys-Arg C-terminal pre-pro-polypeptide of *S. cerevisiae* α-factor gene, and a DNA sequence encoding a mature protein heterologous to the yeast organism, wherein the sequences are joined directly together and do not include Glu (or Asp)-Ala repeats.

10. In addition to describing the above α-factor/EGF construct, I also presented results showing the use of that construct to transform a yeast organism, culture that organism, and recover mature EGF therefrom.

11. One skilled in the art, having attended my poster session and presentation on April 29, 1983, would have been able

to make and use the spacerless α-factor/EGF construct described above, or such constructs using a g ne other than that for EGF.

12. I have also read the pap r by Hitz man et al., Science (1983) 219:620-625, and understand its contents. Hitzeman et al. showed that it was possible to express human interferon in yeast. As one skilled in the art, I would have found it obvious to use the human interferon gene of Hitzeman et al. in the α-factor construct I disclosed at the Keystone Conference to obtain the invention of claims 20 and 21 of Singh. It would have been obvious to replace the human EGF gene disclosed by me with the human interferon gene of Hitzeman et al. to arrive at the invention of claims 20 and 21, since Hitzeman et al. clearly suggest the desirability of making interferon in yeast.

13. There are dozens of genera and tens of thousands of species of yeast. Yeast is a diverse group of microorganisms, most species of which are relatively poorly understood. Of these tens of thousands of species, only one species outside the genus *Saccharomyces*, namely *Kluyveromyces lactis*, is known to produce an α-factor related peptide. Nothing in the Singh application would adequately guide one skilled in the art to determine which yeast, other than the specifically disclosed *Saccharomyces* yeast, possess the disclosed characteristics and utility.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements

were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

3/1/92
Date

Anthony J. Brake

gldm040C

CERTIFICATE OF SERVICE

It is hereby certified that a copy of the foregoing DECLARATION OF ANTHONY J. BRAKE has been served by Express Mail upon the attorneys of record for the party Singh to this interference, on this 2nd day of March, 1992, at the following address:

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Atty Dkt 22300-20006.30

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

BRAKE	:	Interference No. 102,728
v.	:	
SINGH	:	Ronald H. Smith Examiner-in-Chief

BRAKE EXHIBITS ACCOMPANYING PRELIMINARY MOTIONS AND DECLARATIONS

Box Interference
Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Brake submits the following Exhibits 1 through 20 in connection with the Preliminary Motions and Declarations filed in the above-referenced Interference. These exhibits are referenced in the various motions and declarations and are submitted once in this form so as to simplify the record:

1. Brake U.S. Patent No. 4,870,008;

2. List of Attendees to 12th Annual UCLA Symposia held in Keystone, Colorado;

3. Hitzeman et al. Science: (1983) 219:620-625;

4. Papers Nos. 10 and 14 of Chang prosecution U.S. Serial No. 488,337 (matured as Chang et al., U.S. Patent No. 5,010,003);

5. Paper No. 16, pages 11-14, of Chang prosecution U.S. Serial No. 488,337 (matured as Chang et al., U.S. Patent No. 5,010,003);

6. Paper No. 35 of Chang prosecution U.S. Serial No. 488,337 (matured as Chang et al., U.S. Patent No. 5,010,003);

7. Curriculum Vitae of Dr. Patricia Tekamp-Olson;

8. Curriculum Vitae of Dr. Guy Mullenbach;

9. Page 12 of Brake 1, U.S. Serial No. 457,325;

10. H. Gregory and B.M. Preston, Int. J. Peptide Protein Res 9: 107-118 (1977);

11. Preliminary Amendment filed concurrently with Brake 3, U.S. Serial No. 081,302;

12. Paper No. 5 (Interference Request) of Singh U.S. Serial No. 552,719 (Singh 3);

13. Form PTO-436 from Brake 1 showing 1/12/83 filing date with Preliminary Amendment; Form PTO 436-from Brake 2 showing 8/12/83 filing date; 5/14/84 Telephone Restriction of Brake 1; and first Office Action, dated 6/27/84 of Brake 1;

14. Ex Parte Singh, Paper No. 29 of U.S. Serial No. 06/506,098 (Singh 2);

15. Paper No. 18 (Examiner Interview Summary) of Brake U.S. Serial No. 522,909;

16. Pap r No. 19 (Amendment) of Brake U.S. Serial No. 522,909;

17. Hitzeman U.S. Patent No. 4,775,622;

18. U.S. Serial No. 06/457,325 (Brake 1) -- served upon Attorneys of Record for Party Singh only;

19. U.S. Serial No. 06/522,909 (Brake 2) -- served upon Attorney of Record for Party Singh only; and

20. Curriculum Vitae of Dr. Anthony Brake.

Respectfully submitted,

By: Thomas E. Ciotti

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Registration No. 21,013

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Journal of Cellular Biochemistry
Formerly Journal of Supramolecular Structure and Cellular Biochemistry
SUPPLEMENT 7B, 1983

12th Annual

UCLA SYMPOSIA

Abstracts

MARCH 27 – APRIL 30, 1983

Alan R. Liss, Inc., New York

Brake Exhibit 2
Brake v. Singh
Interference No. 102,728

8-448946

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Atty Dkt 22300-20006.30

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

BRAKE	:	Interference No. 102,728
v.	:	
SINGH	:	Ronald H. Smith Examiner-in-Chief

MOTION (4) BY THE PARTY BRAKE PURSUANT TO 37 C.F.R. § 1.633(a) FOR JUDGMENT ON THE GROUND THAT THE CLAIMS OF PARTY SINGH ARE UNPATENTABLE UNDER 35 U.S.C. §§ 102(a) AND 103

Box Interference
Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I. STATEMENT OF RELIEF REQUESTED

The party Brake (hereinafter "Brake") hereby moves, pursuant to the provisions of 37 C.F.R. § 1.633(a), for judgment on the grounds that: (a) claims 8 and 19 of the application U.S. Serial No. 07/552,719 (hereinafter "Singh 3"), of party Singh ("Singh") are unpatentable under 35 U.S.C. § 102(a), based on the public knowledge of the invention of those claims on April 29, 1983, which, on the present record, is before the invention thereof by

Singh; and (b) claims 20 and 21 of the application of Singh are unpatentabl under 35 U.S.C. § 103, based on the aforementioned public knowledg , further in view of Hitz man et al., Science (1983) 219:620-625 (submitted herewith as Exhibit 3).

II. STATEMENT OF FACTS AND LAW IN SUPPORT OF MOTION

A. Claims 8 and 19.

35 U.S.C. § 102 provides in part that a person shall be entitled to a patent unless --

> a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent.

Anticipatory knowledge under § 102(a) means public knowledge of a complete and operative invention, Rosemount, Inc. v. Beckman Instruments, Inc., 218 U.S.P.Q. 881 (C.D. Cal. 1983), aff'd, 221 U.S.P.Q. 1 (Fed. Cir. 1984), including each and every element of the claimed invention. In re Bond, 15 U.S.P.Q.2d 1566, 1567 (Fed. Cir. 1990).

Claim 8 of the Singh application reads:

> A yeast expression vehicle comprising the DNA sequence encoding a lys arg C-terminal pre-pro peptide of yeast alpha factor gene operably connected in translation reading frame without intervening Glu (or Asp)-Ala dipeptide repeats to a DNA sequence encoding a mature protein heterologous to the yeast organism, wherein the DNA encoding all of the Glu (or Asp)-Ala dipeptide repeats has been deleted from the pre-pro peptide of the yeast alpha factor DNA.

Thus the elements of claim 8 are:

> A yeast expression vehicle which comprises:
>
> (a) a DNA sequence encoding a Lys-Arg C-terminal pre-pro peptide of yeast alpha factor gene;
> (b) a DNA sequence encoding a mature protein heterologous to yeast; wherein
>
> (c) the sequences are joined directly together and do not include Glu (or Asp)-Ala repeats.

These elements must be compared with the public knowledge on April 29, 1983. The Declaration of Dr. Anthony Brake (hereinafter "Dr. Brake Decl.", submitted herewith) demonstrates that the invention of claim 8 was disclosed to the public prior to the claimed filing date of claim 8, which is June 20, 1983. (See "Singh Miscellaneous Motion (1) Pursuant to 37 C.F.R. § 1.635" (to Deny Benefit) filed February 26, 1992.)

The 12th Annual UCLA Symposia were held between March 27 and April 30, 1983 in Keystone, Colorado. Dr. Brake Decl. ¶ 7. Many molecular biologists and yeast geneticists, including several Genentech researchers, were in attendance. See List of Attendees, attached hereto as Exhibit 2. On April 29, 1983, Dr. Brake gave a poster session and presentation disclosing a spacerless α-factor construct, such as that in the Count. Dr. Brake Decl. ¶ 7.

On that poster, Dr. Brake presented his results demonstrating the successful construction of a yeast expression vehicle including the DNA construct embodied in claim 8 of the

Singh application. A construct on Dr. Brake's poster consisted of the *S. c revisiae* α-factor leader sequ nc , terminating with the sequence encoding the first Lys-Arg dipeptid , connected in translation reading frame to the sequence encoding the first amino acid of mature epidermal growth factor (EGF). Dr. Brake Decl. ¶ 9.

Thus, the yeast expression vehicle disclosed by Dr. Brake at the Keystone Conference included a DNA construct encoding: (1) a Lys-Arg C-terminal pre-pro peptide of yeast α-factor gene; and (2) a DNA sequence encoding a mature protein heterologous to the yeast organism; wherein (3) the sequences are joined directly together and do not include Glu (or Asp)-Ala repeats. Dr. Brake Decl. ¶ 9. One skilled in the art at that time would have been able to make and use this DNA construct. Id. ¶ 11. As such, Dr. Brake's presentation constituted public knowledge of an invention containing each and every element of claim 8 recited above, and is thus a complete anticipation under 35 U.S.C. § 102 of the invention of that claim. See American Standard, Inc. v. Pfizer, Inc., 14 U.S.P.Q.2d 1673, 1709 & n.42 (D. Del. 1989) (contents of a speech given at a scientific conference in the U.S. constitutes prior art under the public knowledge provision of 35 U.S.C. § 102(a)).

Claim 19 of Singh 3 reads:

> 19. A process for obtaining a mature protein heterologous to yeast as a product of yeast expression, which process comprises:

(a) transforming a yeast organism with an expression vehicle comprising the DNA sequ nce encoding a lys arg C-terminal pre-pro peptide of yeast alpha factor operably connected in translation reading frame without intervening Glu (or Asp)-Ala dipeptide repeats to a DNA sequence encoding a mature protein heterologous to the yeast organism, wherein the DNA encoding all of the Glu (or Asp)-Ala dipeptide repeats has been deleted from the pre-pro peptide of the yeast alpha factor DNA;

(b) culturing the transformed organism; and

(c) recovering mature protein from the culture having an N-terminal amino acid sequence identical to that of the protein from natural sources.

Claim 19 relates to a process for making a mature protein heterologous to yeast involving transforming a yeast organism with the DNA construct of claim 8, culturing the organism, and then recovering the mature protein.

At the Keystone Conference, Dr. Brake, in addition to disclosing the α-factor/EGF construct described above, also disclosed the use of that construct to transform a yeast organism, culture that organism, and recover mature EGF therefrom. Dr. Brake Decl. ¶ 10. Therefore, Dr. Brake's disclosure also anticipated the invention of claim 19.

Thus, claims 8 and 19 read on unpatentable subject matter as defined by 35 U.S.C. § 102(a).[1/]

1/ Dr. Brake's public disclosure does not raise a patentability issue as to the claims in the Brake patent. 35 U.S.C. § 102(a) only bars a patent where the invention was "known or used by

B. Claims 20 and 21.

35 U.S.C. § 103 states, in part:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.

An invention may be obvious over a combination of prior art if there is a teaching or suggestion in the art that would lead one of ordinary skill in the art to make the combination. Smithkline Diagnostics, Inc. v. Helena Laboratories Corp., 8 U.S.P.Q.2d 1468, 1475 (Fed. Cir. 1988).

Claims 20 and 21 in Singh 3 depend upon claims 8 and 19, respectively, and thus contain the elements of those claims, and are further limited to human α-interferon. Dr. Brake's disclosure of April 29, 1983, discussed in detail in Section A, supra, teaches every element of Singh's claims 20 and 21, but does not explicitly teach the specific expression of human α-interferon. However, Dr. Brake's disclosure would have enabled one skilled in the art to make and use the spacerless α-factor construct using a gene other than that for EGF. Dr. Brake Decl. ¶ 11.

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others," not by the inventor himself.

Hitzeman et al., Science (1983) 219:620-625 (Exhibit 3), describes the expression and secretion of human interferon in yeast, and has a publication date of February 11, 1983.[2/] Thus, Hitzeman et al., in combination with Dr. Brake's disclosure, teach all the elements of claims 20 and 21. It would have been obvious to one skilled in the art to use the human interferon gene of Hitzeman et al. in the α-factor system of Brake to obtain the invention of claims 20 and 21. Dr. Brake Decl. ¶ 12. Hitzeman et al. showed that it was desirable to express human interferon in yeast. It would therefore have been obvious to replace the human EGF gene of the Brake disclosure with the human interferon gene of Hitzeman et al. to arrive at the invention of claims 20 and 21. Id. ¶ 12.

Claims 20 and 21 are thus unpatentable under 35 U.S.C. § 103 over Dr. Brake's disclosure in view of Hitzeman et al.

CONCLUSION

For the foregoing reasons, Brake respectfully submits that claims 8 and 19 in party Singh's application are unpatentable under 35 U.S.C. § 102(a), and claims 20 and 21 in party Singh's application are unpatentable under 35 U.S.C. § 103. Brake notes for the record that these grounds of unpatentability do not apply

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2/ In the alternative, Brake also relies on U.S. Patent No. 4,775,622 to Hitzeman et al. (submitted herewith as Exhibit 17), which discloses the same expression and secretion of human interferon in yeast, and has a reference date of November 1, 1982 pursuant to 35 U.S.C. § 102(e).

to Brake because they are based on Brak 's own disclosure which is not prior art to Brake.

Respectfully submitted,

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Registration No. 21,013

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G0405

ABSTRACT

HUMAN BLOOD PLASMA CONTAINS TWO SIMILAR POLYPEPTIDES, INSULIN-LIKE GROWTH FACTORS I AND II (IGF I, 70 AMINO AICDS, AND IGF II, 67 AMINO ACIDS), WHICH COMPRISE A GROUP OF INSULIN-LIKE GROWTH HORMONE-DEPENDENT PEPTIDES WHICH ARE BELIEVED TO MEDIATE THE GROWTH PROMOTING ACTIONS OF GROWTH HORMONE. THE NUCLEOTIDE SEQUENCES CODING FOR THESE POLY-PEPTIDES HAVE BEEN SYNTHESIZED ON SOLID SUPPORT BY A MODIFICATION OF PHOSPHORAMIDITE COUPLING PROCEDURES. SINGLE STRAND SEQUENCES AVERAGING 25 BASES IN LENGTH WERE SYNTHESIZED AND PURIFIED. DUE TO PARTICULARLY LARGE OVERLAPS BETWEEN STRANDS, ASSEMBLY OF EACH OF THESE GENES FROM THEIR OLIGOMERS WAS ACHIEVED IN A SINGLE ANNEALING AND LIGATION EXPERIMENT WITHOUT THE PIECEMEAL ASSEMBLY APPROACH CONVENTIONALLY REPORTED. CODONS CHOSEN FOR THESE SYNTHESES FOLLOWED THE FOLLOWING PRINCIPLES: (1) CODONS USING THE YEAST CODON BIAS WERE SELECTED TO MAXIMIZE EXPRESSION IN THIS ORGANISM. (2) RESTRICTION SITES WERE BUILT INTO THE GENES AT CONVENIENT LOCATIONS IN ORDER TO ALLOW FOR CONSTRUCTION OF 6 DIFFERENT IGF I/IGF II GENE HYBRIDS AND POLYPEPTIDE HYBRID MOLECULES. YEAST CELLS TRANSFORMED WITH PLASMIDS CONTAINING THESE GENES PRODUCED BIOLOGICALLY ACTIVE IGF I AND IGF II.

Barr Et Al Exhibit I
Lee Et Al. V Barr Et Al.
Interference No. 102, 208

INTRODUCTION

IT IS SUSPECTED THAT SOMATIC GROWTH WHICH FOLLOWS THE ADMINISTRATION OF GROWTH HORMONE IN VIVO IS MEDIATED THROUGH A FAMILY OF MITOGENIC, INSULIN-LIKE PEPTIDES WHOSE SERUM CONCENTRATIONS ARE GROWTH HORMONE DEPENDENT. AMONG THESE PEPTIDES INSULIN-LIKE GROWTH FACTORS I AND II HAVE BEEN ISOLATED IN LIMITED AMOUNTS FROM HUMAN SERUM AND SEQUENCED.[1,2]

IT HAS BEEN OF PARTICULAR SCIENTIFIC AND CLINICAL INTEREST TO US TO PRODUCE RELATIVELY LARGE QUANTITIES OF THESE GROWTH FACTORS. TO THIS END WE PRESENT HERE (1) CHEMICAL TECHNIQUES FOR THE SYNTHESIS OF GENES CODING FOR THESE GROWTH FACTORS AND (2) RECOMBINANT DNA TECHNIQUES WHICH UTILIZE A YEAST/a-FACTOR EXPRESSION SYSTEM THAT ACHIEVES SECRETION OF THESE PROTEINS FROM YEAST.

RINDERKNECKT & HUMBEL, J. BIOL. CHEM., (1978).
RINDERKNECKT & HUMBEL, FEBS LETTERS, (1978).

METHODS

DESIGN

THE CODON SEQUENCES OF SYNTHETIC GENES CODING FOR IGF-I AND II WERE ESTABLISHED BY UTILIZING THEIR PUBLISHED PROTEIN SEQUENCES[1,2] (FIGURE 1).

CODONS WERE SELECTED SUCH THAT:

(1) EXPRESSION IN YEAST MIGHT BE MAXIMIZED BY UTILIZING THOSE CODONS MOST FREQUENTLY FOUND IN THE GLYCOLYTIC ENZYMES OF YEAST (i.e. BY MAINTAINING THE YEAST CODON BIAS).

(2) ASSEMBLIES WERE FACILITATED BY REMOVAL OF LONG HOMOLOGOUS STRETCHES WHICH MIGHT CAUSE INCORRECT ANNEALING OF OLIGOMERS.

(3) CONVENIENT RESTRICTION SITES WERE GENERATED SO THAT VARIOUS HYBRID IGF-I/IGF-II GENE AND POLYPEPTIDE CONSTRUCTIONS CAN BE SYNTHESIZED.

(4) UNDESIRABLE RESTRICTION SITES WERE REMOVED.

OLIGOMERIC COMPONENTS WERE SYNTHESIZED SO AS TO YIELD MAXIMUM OVERLAPS AND TO MAKE MOST EFFICIENT USE OF SEGMENTS HOMOLOGOUS TO BOTH GENES (FIGURE 3).

DNA SYNTHESIS

OLIGOMERS AVERAGING 25 BASES IN LENGTH (FIGURE 2) WERE SYNTHESIZED ON A SOLID SUPPORT BY A PHOSPHORAMIDITE COUPLING APPROACH.

ASSEMBLIES

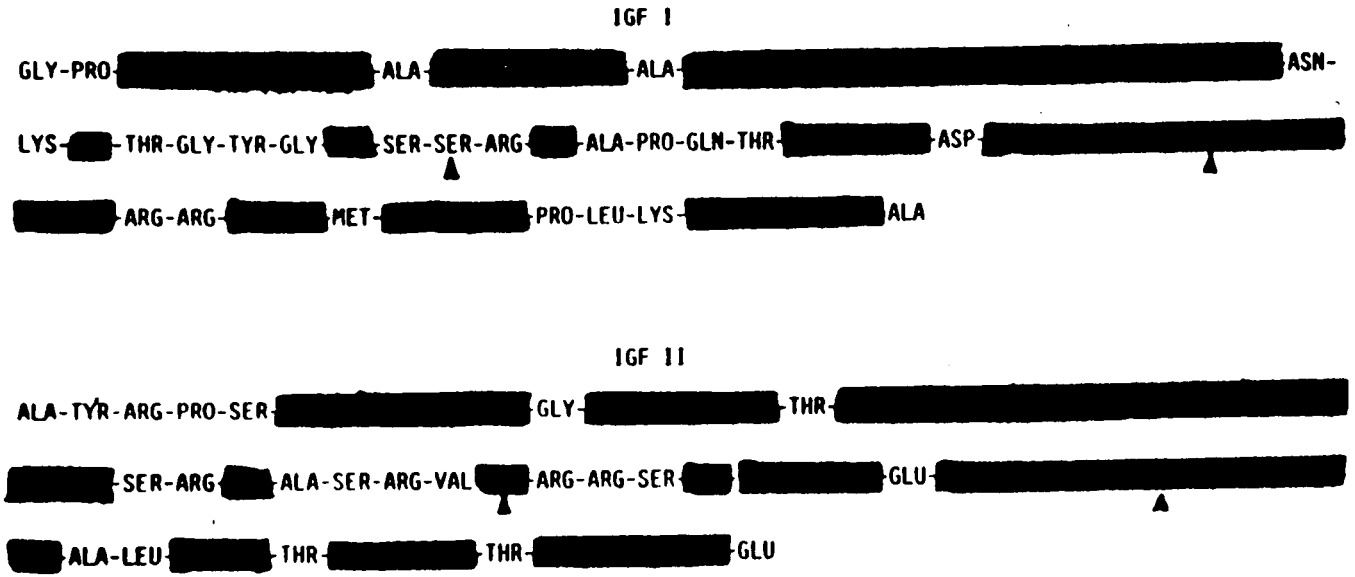
THE ENZYMATIC LIGATION OF EACH GENE WAS ACHIEVED IN A SINGLE ANNEALING/LIGATION POOL RATHER THAN BY WAY OF PIECEMEAL ASSEMBLIES USUALLY REPORTED (FIGURE 3). CLONED CONSTRUCTIONS WERE SEQUENCED BY THE MAXAM GILBERT PROCEDURE (FIGURE 4).

EXPRESSION

PROTEIN CODING REGIONS WERE DIRECTLY FUSED TO THE YEAST α-FACTOR LEADER CODING REGION IN SUCH A WAY THAT THE REGIONS CODING FOR THE α-FACTOR PROCESSING SITES ARE MAINTAINED (FIGURE 5). YEAST CELLS TRANSFORMED WITH SUCH PLASMID CONSTRUCTS APPEAR TO SECRETE IGF-I OR IGF-II. PRELIMINARY RESULTS FROM RADIOIMMUNOASSAYS (BY MARTIN SPENCER, CHILDREN'S HOSPITAL, S.F.), RECEPTOR BINDING ASSAYS (M. SPENCER), A BIOASSY (PIGEON CROP GROWTH ACTIVITY), AND MOLECULAR WEIGHT DATA (FIGURE 6) SUPPORT THE IDENTITIES OF THESE HORMONES.

FIGURE 1.

FIGURE 1. PROTEIN SEQUENCES OF IGF I[1] AND II[2]

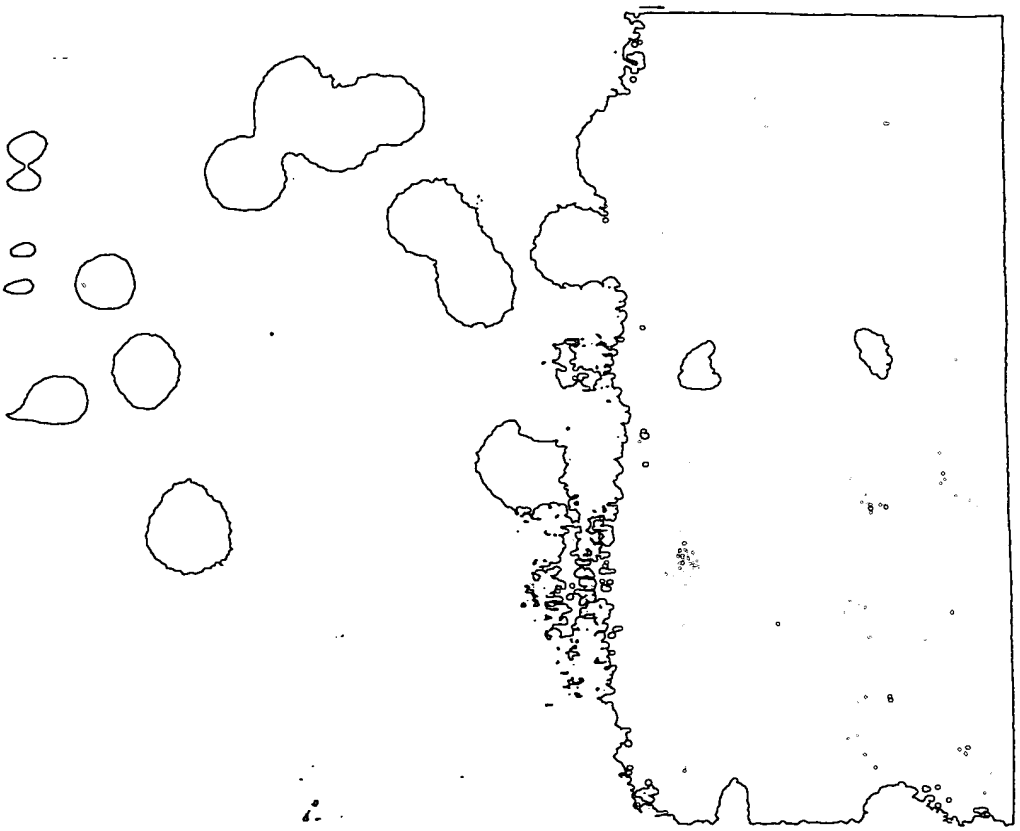


HOMOLOGOUS PORTIONS SHOWN IN YELLOW.

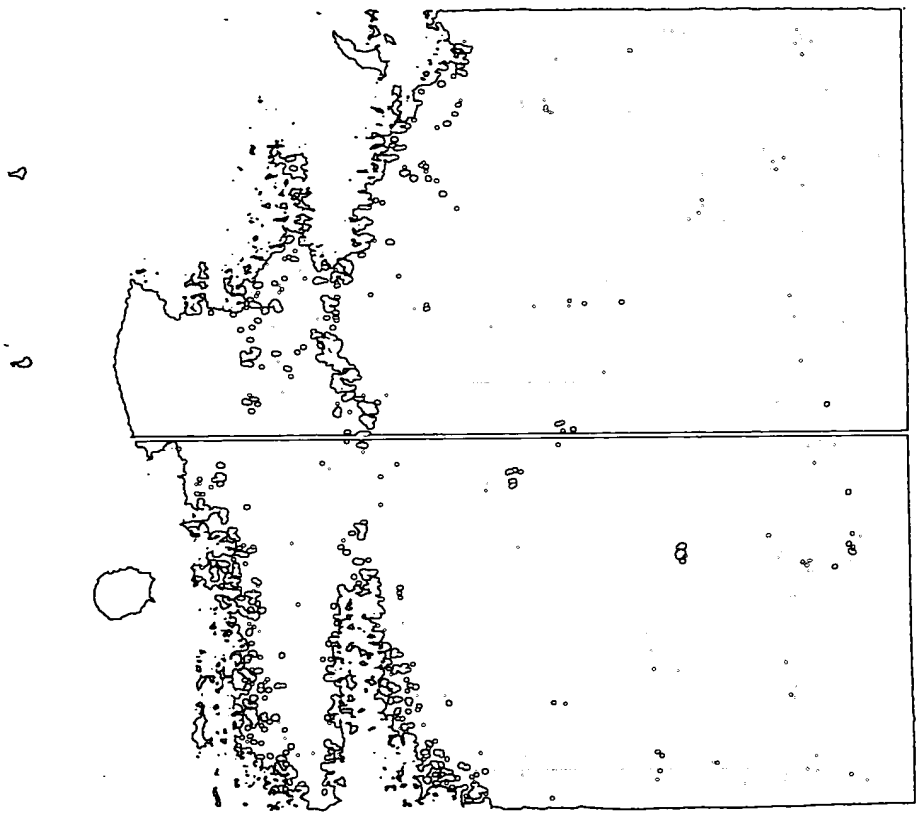
1. RINDERKNECHT & HUMBEL, J. BIOL. CHEM., (1978).

2. RINDERKNECHT & HUMBEL, FEBS LETTERS, (1978).

IGF I



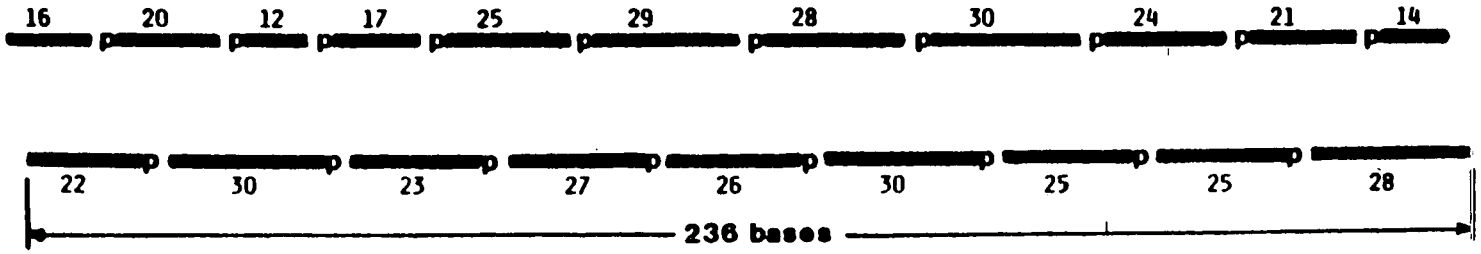
IGF II



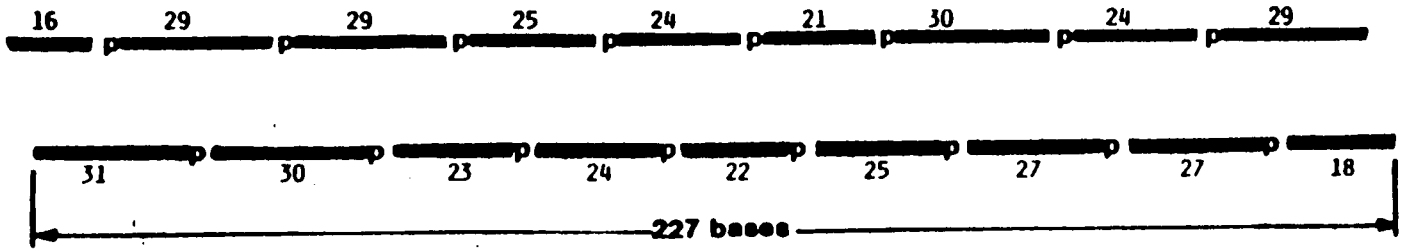
THESE OLIGONUCLEOTIDES AVERAGING 25 BASES IN LENGTH WERE SYNTHESIZED ON A SOLID SUPPORT BY A PHOSPHORAMIDITE COUPLING PROCEDURE (URDEA, MERRYWEATHER, MULLENBACH, COIT, HEDERLEIN, VALENZUELA & BARR, PNAS, SUBMITTED FOR PUBLICATION) AND SIZED BY POLYACRYLAMIDE GEL ELECTROPHORESIS.

FIGURE 3. ASSEMBLY SCHEME

IGF-I



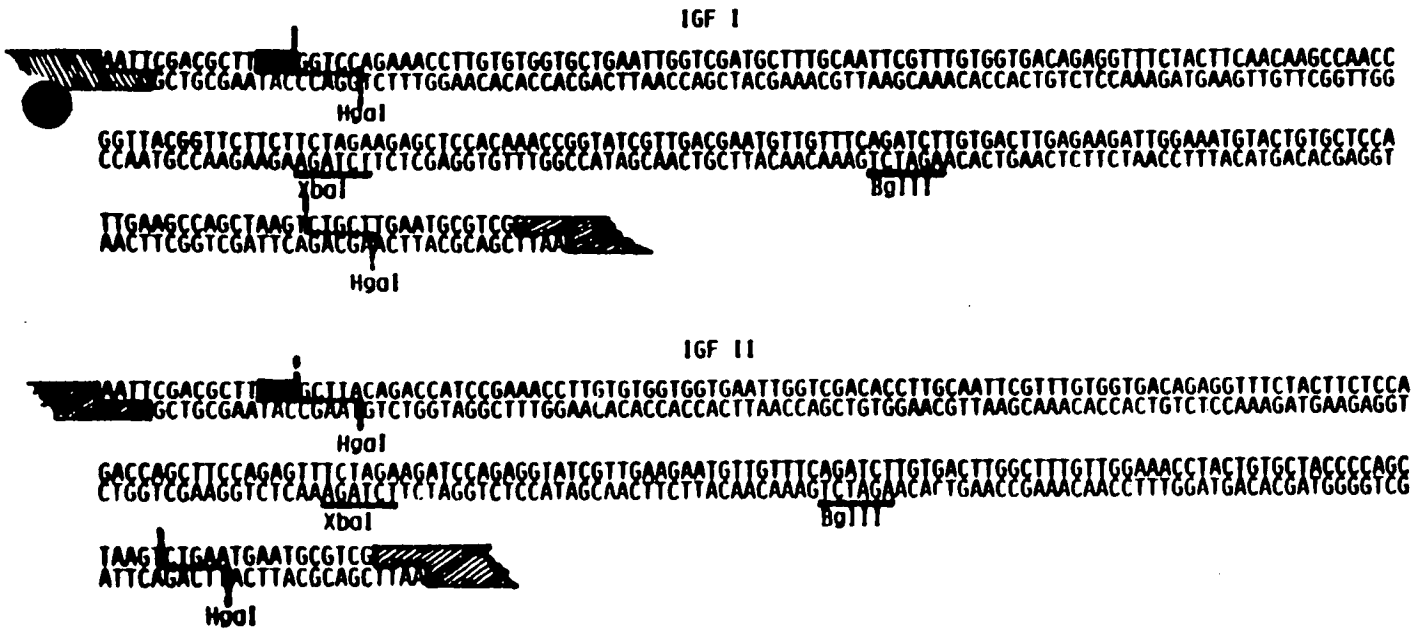
IGF-II



LARGE OVERLAPS BETWEEN STRANDS OF ABOUT 25 BASES LONG PERMIT ASSEMBLY IN A SINGLE ANNEALING AND LIGATION POOL RATHER THAN PIECEMEAL ASSEMBLIES PREVIOUSLY REPORTED.

FIGURE 4.

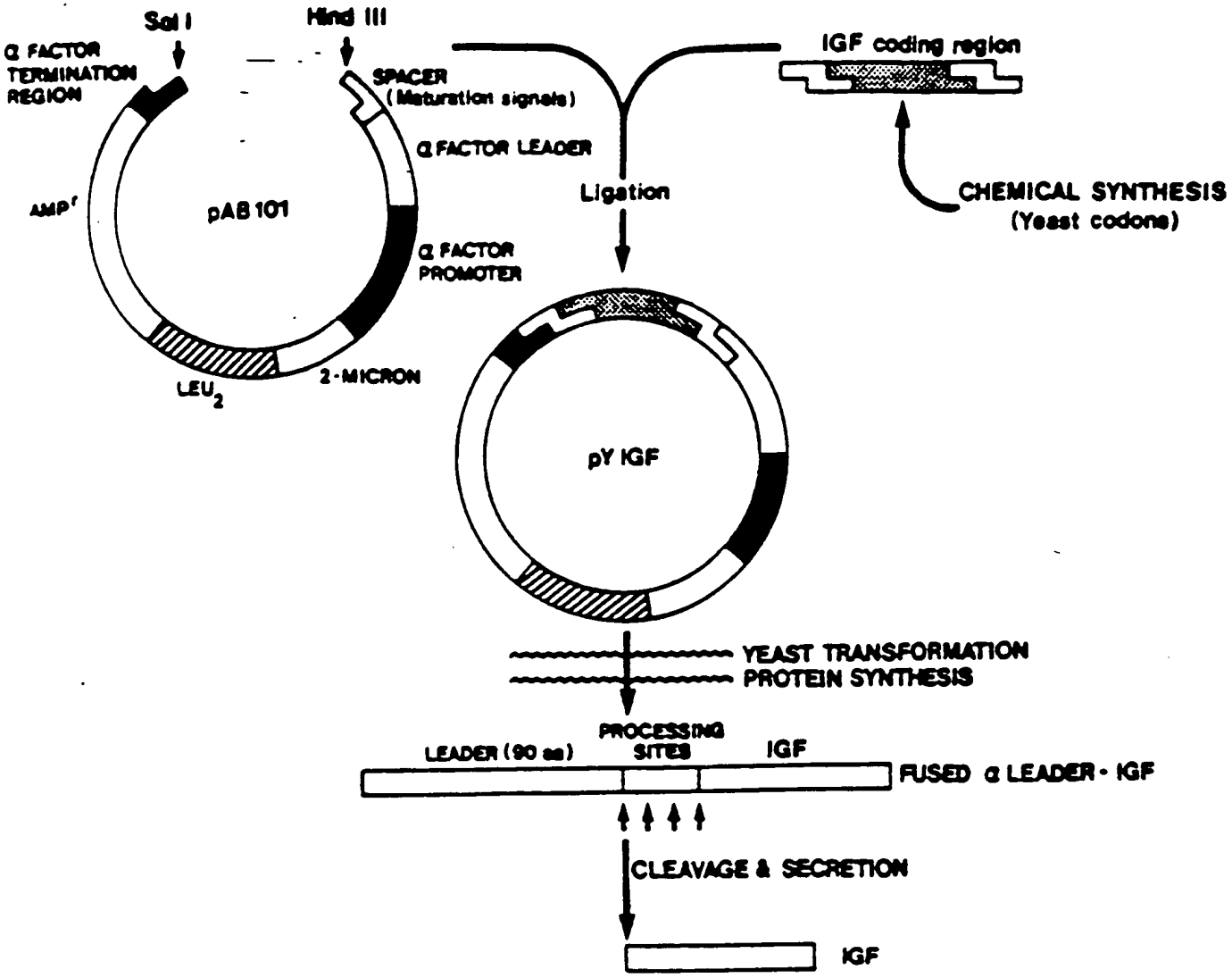
FIGURE 4. DNA SEQUENCING RESULTS



THE SYNTHETIC DOUBLE-STRANDED CONSTRUCTIONS WERE CLONED INTO EcoRI DIGESTED pBR328 AND THE INSERT SEQUENCED BY THE MAXAM AND GILBERT PROCEDURE.

RESTRICTION SITES IN RED HAVE BEEN BUILT INTO THE GENE FOR POTENTIAL HYBRID IGF I/IGF II CONSTRUCTIONS.

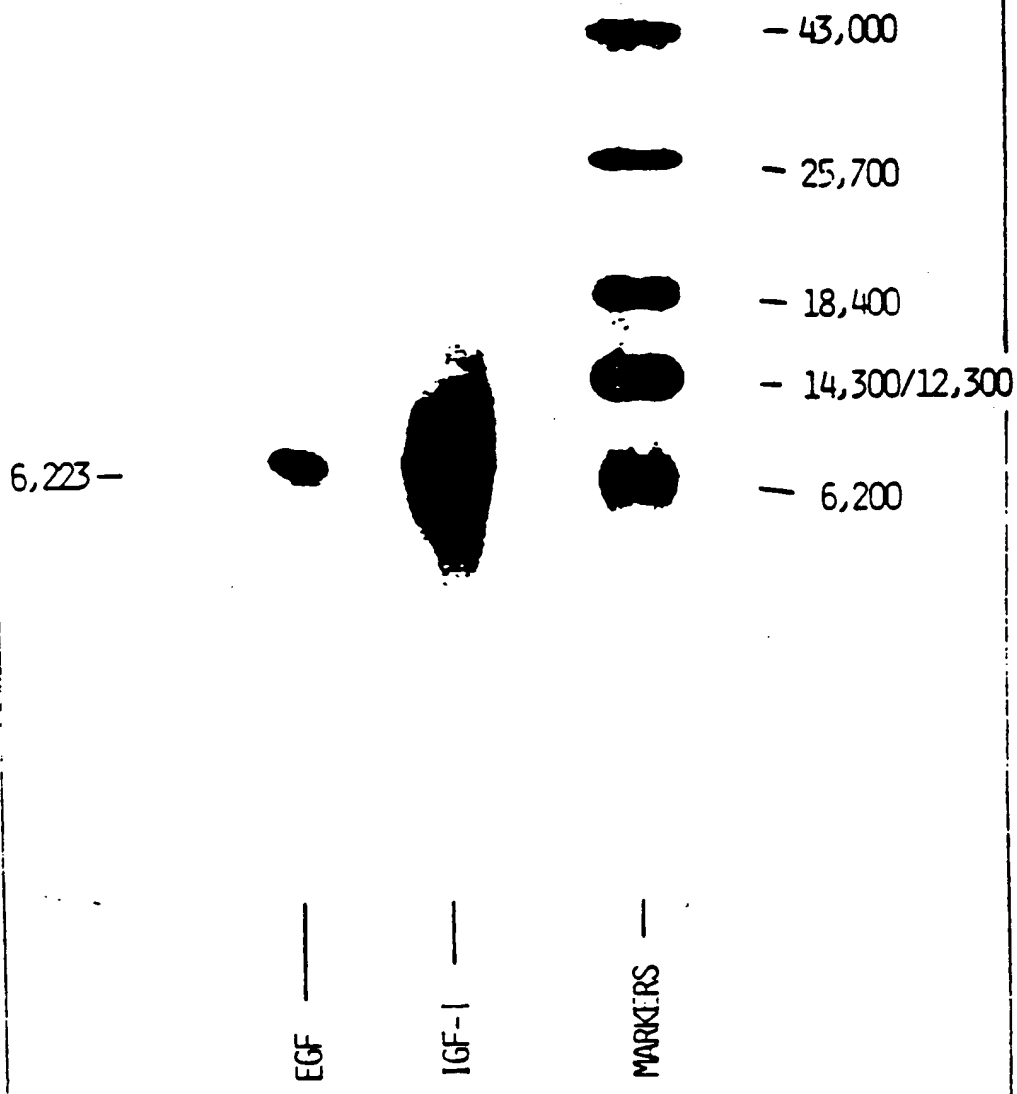
FIGURE 5. PROCESSING AND SECRETION OF PROTEINS IN YEAST



FIRST THE CODING SEQUENCE TO BE CLONED INTO YEAST WAS PRECISELY EXCISED FROM THE pBR328/IGF PLASMID WITH HgaI (WHICH CUTS OUTSIDE ITS RECOGNITION SITE). THIS FRAGMENT WAS THEN EQUIPPED WITH LINKERS SUCH THAT THE REGIONS CODING FOR THE α-FACTOR PROCESSING SITES ARE MAINTAINED, AND THEN CLONED INTO THE YEAST α-FACTOR VECTOR. APPROPRIATE POSTTRANSLATIONAL PROCESSING IS ACHIEVED UPON SECRETION OF IGFs FROM YEAST.

FIGURE 6.

FIGURE 6. SDS POLYARCRLAMIDE GEL ELECTROPHORESIS



ONLY THE YEAST CULTURE SUPERNATANT WAS SUBMITTED TO BIOREX-70 CATION EXCHANGE CHROMATOGRAPHY PRIOR TO SDS GEL ELECTRO-PHORESIS.

CONCLUSIONS

GENES CODING FOR HUMAN INSULIN-LIKE GROWTH FACTORS I AND II HAVE BEEN SYNTHESIZED BY CHEMICAL MEANS. EACH WAS ABLE TO BE ASSEMBLED IN A SINGLE POOL FROM THEIR OLIGOMERIC COMPONENTS. IGF-I AND IGF-II ARE EXPRESSED AND SECRETED IN YEAST AT LEVELS AS HIGH AS ABOUT 1 MG/LITER OF CELL CULTURE (IGF-I). THUS, FOR THE FIRST TIME, SUFFICIENT QUANTITIES OF THESE PROTEINS ARE AVAILABLE FOR EXTENSIVE STUDY.